## SCIENTIFIC ABSTRACT

Patients with glioblastoma have elevated levels of Transforming Growth Factor-ß2 (TGF-ß2) which is associated with immunosuppression. TGF-ß2 suppresses T-cells activation in part by inhibiting high affinity IL-2 receptors. Like human gliomas, rat 9L gliosarcoma cells secrete active TGF-ß and intracranial implantation of as few as 300 of these tumor cells in Fisher-344 rats results in over 99% fatality after five weeks.

We hypothesized that genetic modification of glioma cells to block TGF-ß2 expression would result in the gene modified cells becoming more immunogenic and suitable for active tumor immunotherapy. We demonstrated the efficacy of this approach by immunizing rats bearing intracranial tumors with IL-2 and / or TGF-ß2 antisense gene modified tumor cells. In animal studies 5 $x10^3$ unmodified 9L cells were implanted into the forebrain of Fisher-344 rats and immunizations were initiated four days later. Animals received a total of 4 subcutaneous immunizations on a twice a week schedule with 2.5 x $10^5$ gene modified tumor cells. As a control one group of animals did not receive any immunizations. All control animals died within five weeks because of growing tumors. Subcutaneous immunizations of tumor bearing animals with TGF-ß2 antisense or IL-2 and TGF-ß2 antisense gene modified 9L cells respectively resulted in 17 out of 17 and 7 out of 7 tumor free animals 12 weeks post tumor implantation. In contrast, immunization of animals with IL-2 gene modified or empty vector gene modified 9L cells resulted in 3 out of 10 and 2 out of 15 tumor-free animals, respectively. Histologic assessment of these brains confirmed the lack of tumor formation in the surviving animals.

Surviving animals from different experiments were subjected to secondary intracranial tumor challenges in the contralateral hemisphere with $10^5$ or $10^6$ parental tumor cells. All animals tolerated the secondary tumor challenge with $10^5$ unmodified tumor cells and did not develop tumors for a six month observation period. In the secondary intracranial tumor challenge with $10^6$ unmodified 9L cells, the animals that were immunized with vector modified or IL-2 gene modified 9L cells died by three weeks post tumor implantation. In contrast, 10 out of 11 animals inoculated with one of the TGF-ß gene modified tumor cell lines survived more than 4 weeks (see Preclinical Studies, Appendix 12.7 for details).

*In vitro* cytotoxicity assays using lymph node effector cells taken from immunized animals and activated *in vitro* in the presence of irradiated tumor cells and 50 BRMP units of IL-2 revealed a 4-to-8 fold increase in lytic activity for the animals immunized with TGF-ß2 antisense gene modified tumor cells with or without IL-2 gene modification when compared to the lytic activity for the animals immunized with vector or IL-2 gene modified tumor cells.

These results warrant the evaluation of this gene therapy approach in a Phase I Clinical Trial. In this trial, tumor samples will be obtained from the patients at the time of clinically indicated surgery. These tumors will be grown in culture to establish a cell line for each patient. The patients' tumor cells will be genetically altered with a human TGF-ß2 antisense vector to inhibit their secretion of TGF-ß. Following completion of the traditional post-surgical radiation therapy, the first group of patients will receive, in approximately three week intervals, four injections of 5 x $10^6$ irradiated gene modified autologous tumor cells. Subsequently, in dose escalation studies the second group of patients will receive injections of 1 x $10^7$, and the third group will receive injections of 2 x $10^7$ irradiated TGF-ß2 gene modified tumor cells. The results of this Phase I trial will be used to assess the safety of this form of gene therapy and may also provide preliminary data to evaluate the potential utility of TGF-ß2 antisense gene therapy in management of glioma tumors.